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Sequence Listing could not be accepted due to errors.

See attached Validation Report.

If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).

Reviewer: Anne Corrigan

Timestamp: [year=2008; month=1; day=23; hr=13; min=45; sec=43; ms=375; ]

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\*\*\*\*\*

Reviewer Comments:

<120> A METHOD FOR CREATING A FUNCTIONALLY ACTIVE CHIMERIC TYPE IIG  
RESTRICTION ENDONUCLEASE (as amended)

The first line of the above <120> response exceeds the Sequence Rules' required 72-character line (this includes white spaces). Please insert a hard return after "TYPE" on the first line, and align the second line.

(from Sequence 2)

Val	Asp	Glu	Ala	Leu	Leu	Ile	Lys	Tyr	His	Gly	Phe	Ser	Glu	Lys	Glu
				515						520					525

Val	Lys	Gln	Leu	Arg	Gly	Ile	Trp	Lys	Lys	Leu	Ser	Gln	Arg	Arg	Asn
				530						535					540

Asn Arg Thr Lys Lys  
545

Please delete the excess blank lines above: only one blank line should separate each amino acid line.

<210> 38

<211> 103

<212> PRT

<213> artificial

<220>

<223> segment of protein sequence of catechol O-methyltransferase

<400> 39

Please change the above <210> response to "39." <210> 38 was already shown.

\*\*\*\*\*

Application No: 10800946

Version No: 2.0

**Input Set:****Output Set:****Started:** 2008-01-09 14:09:09.990**Finished:** 2008-01-09 14:09:12.054**Elapsed:** 0 hr(s) 0 min(s) 2 sec(s) 64 ms**Total Warnings:** 25**Total Errors:** 2**No. of SeqIDs Defined:** 43**Actual SeqID Count:** 43

Error code	Error Description
W 213	Artificial or Unknown found in <213> in SEQ ID (19)
W 213	Artificial or Unknown found in <213> in SEQ ID (20)
W 213	Artificial or Unknown found in <213> in SEQ ID (21)
W 213	Artificial or Unknown found in <213> in SEQ ID (22)
W 213	Artificial or Unknown found in <213> in SEQ ID (23)
W 213	Artificial or Unknown found in <213> in SEQ ID (24)
W 213	Artificial or Unknown found in <213> in SEQ ID (25)
W 213	Artificial or Unknown found in <213> in SEQ ID (26)
W 213	Artificial or Unknown found in <213> in SEQ ID (27)
W 213	Artificial or Unknown found in <213> in SEQ ID (28)
W 213	Artificial or Unknown found in <213> in SEQ ID (29)
W 213	Artificial or Unknown found in <213> in SEQ ID (30)
W 213	Artificial or Unknown found in <213> in SEQ ID (31)
W 213	Artificial or Unknown found in <213> in SEQ ID (32)
W 213	Artificial or Unknown found in <213> in SEQ ID (33)
W 213	Artificial or Unknown found in <213> in SEQ ID (34)
W 213	Artificial or Unknown found in <213> in SEQ ID (35)
W 213	Artificial or Unknown found in <213> in SEQ ID (36)
W 213	Artificial or Unknown found in <213> in SEQ ID (37)
W 213	Artificial or Unknown found in <213> in SEQ ID (38)

**Input Set:**

**Output Set:**

**Started:** 2008-01-09 14:09:09.990  
**Finished:** 2008-01-09 14:09:12.054  
**Elapsed:** 0 hr(s) 0 min(s) 2 sec(s) 64 ms  
**Total Warnings:** 25  
**Total Errors:** 2  
**No. of SeqIDs Defined:** 43  
**Actual SeqID Count:** 43

Error code	Error Description
	This error has occurred more than 20 times, will not be displayed
E 212	Invalid Sequence ID Number; Expected 39 as next SeqID but skipped
E 212	Invalid Sequence ID Number; Expected 39 as next SeqID but skipped

## SEQUENCE LISTING

<110> Xu, Shuang-yong  
Kobbe, Daniela  
Zhu, Zhenyu  
Samuelson, James

<120> A METHOD FOR CREATING A FUNCTIONALLY ACTIVE CHIMERIC TYPE IIG RESTRICTION ENDONUCLEASE  
(as amended)

<130> NEB-183-CIP

<140> 10800946

<141> 2004-03-15

<150> 10/150,028

<151> 2002-05-17

<150> 09/693,146

<151> 2000-07-02

<160> 43

<170> PatentIn version 3.2 (1-26) and 3.4 (27-43)

<210> 1

<211> 1650

<212> DNA

<213> Bacillus pumilus

<220>

<221> CDS

<222> (1) .. (1650)

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1 5 10 15	

tat tac acc cct aaa gtt att gct gac ttt tta tgt caa tgg agt att	96
Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile	
20 25 30	

caa gat gac aca aag agt gta ctt gaa ccc agt tgt gga gat ggt aat	144
Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn	
35 40 45	

ttt att gaa tcg gca ata ctt agg ttc aaa gaa ctt agt ata gat aat	192
Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn	
50 55 60	

gaa caa ctt aaa gga aga att aca gga gta gag cta att gaa gaa gaa	240
Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu	
65 70 75 80	

gct ttg aaa gtt caa aat cga gca aat gag ttg ggg gtt gat aaa aac	288
Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn	

tca ata gta aat agt gac ttc ttt caa ttt gta aaa gat aat aag aat	336
Ser Ile Val Asn Ser Asp Phe Phe Gln Phe Val Lys Asp Asn Lys Asn	
100 105 110	
aaa aaa ttt gat act att att ggt aat cca cca ttc ata aga tac caa	384
Lys Lys Phe Asp Thr Ile Ile Gly Asn Pro Pro Phe Ile Arg Tyr Gln	
115 120 125	
aac ttt cct gaa gag cat cgt agt ata gcc atg gaa atg atg gag gaa	432
Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu	
130 135 140	
cta ggt tta aaa cct aat aaa ctt aca aat atc tgg gtt cca ttt cta	480
Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu	
145 150 155 160	
gtg gta tct gct aca tta ctt aat gaa caa gga aag atg gct atg gtt	528
Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val	
165 170 175	
ata ccg gct gaa tta ttt cag gta aag tat gca gca gaa aca aga att	576
Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile	
180 185 190	
ttt tta tca aag ttt ttc gat cgt atc act ata att aca ttt gaa aaa	624
Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys	
195 200 205	
ctt gtt ttt gaa aat atc caa cag gaa gtt ata cta ctt ctt tgt gaa	672
Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Leu Cys Glu	
210 215 220	
aag aaa gtt aat aaa ggt aaa gga att cgg gtt att gaa tgc gag aac	720
Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn	
225 230 235 240	
tta gat gga tta aat tcc att gat ttt gta gct ata aat ggt tca aat	768
Leu Asp Gly Leu Asn Ser Ile Asp Phe Val Ala Ile Asn Gly Ser Asn	
245 250 255	
gtt aaa cct att gaa cac cgt act gaa aag tgg aca aag tat ttc tta	816
Val Lys Pro Ile Glu His Arg Thr Glu Lys Trp Thr Lys Tyr Phe Leu	
260 265 270	
aac gaa gat gaa ata ctt ctt tta cag agt tta aag gaa gac aaa cgc	864
Asn Glu Asp Glu Ile Leu Leu Leu Gln Ser Leu Lys Glu Asp Lys Arg	
275 280 285	
gtt aaa aat tgt aat gac tat ttt aag aca gaa gtt ggc tta gtt act	912
Val Lys Asn Cys Asn Asp Tyr Phe Lys Thr Glu Val Gly Leu Val Thr	
290 295 300	
gga cga aac gaa ttc ttt atg atg aaa gaa aac caa gta aaa gaa tgg	960
Gly Arg Asn Glu Phe Phe Met Met Lys Glu Asn Gln Val Lys Glu Trp	

305	310	315	320	
aat cta gaa gaa tat	aca ata cct gtt	aca ggt agg tcc	aat cag tta	1008
Asn Leu Glu Glu Tyr	Thr Ile Pro Val	Thr Gly Arg Ser	Asn Gln Leu	
325	330	335		
aaa ggt ata aca ttt	aca gaa aat gat	ttt cat gaa aat	tca atg gaa	1056
Lys Gly Ile Thr Phe	Thr Glu Asn Asp	Phe His Glu Asn	Ser Met Glu	
340	345	350		
caa aag gca att cac	cta ttt ttg cca	cca gat gaa gat	ttt gaa aag	1104
Gln Lys Ala Ile His	Leu Phe Leu Pro	Pro Asp Glu Asp	Phe Glu Lys	
355	360	365		
tta ccg att gag tgt	caa aat tat atc	aag tat ggg gaa	gaa aaa ggc	1152
Leu Pro Ile Glu Cys	Gln Asn Tyr Ile	Lys Tyr Gly Glu	Glu Lys Gly	
370	375	380		
ttc cat caa ggc tat	aaa acc aga att	aga aaa cgt tgg	tat ata act	1200
Phe His Gln Gly Tyr	Lys Thr Arg Ile	Arg Lys Arg Trp	Tyr Ile Thr	
385	390	395	400	
cca tct aga tgg gtt	cca gat gct ttt	gct tta aga cag	gtt gat ggc	1248
Pro Ser Arg Trp Val	Pro Asp Ala Phe	Ala Leu Arg Gln	Val Asp Gly	
405	410	415		
tat cca aaa cta att	tta aat gaa acc	gac gct tct tct	act gat aca	1296
Tyr Pro Lys Leu Ile	Leu Asn Glu Thr	Asp Ala Ser Ser	Thr Asp Thr	
420	425	430		
att cat agg gtt aga	ttt aaa gaa ggt	ata aat gaa aag	tta gcc gta	1344
Ile His Arg Val Arg	Phe Lys Glu Gly	Ile Asn Glu Lys	Leu Ala Val	
435	440	445		
gtt tca ttt ttg aac	tca ctc act ttt	gca tct tca gaa	ata acg ggg	1392
Val Ser Phe Leu Asn	Ser Leu Thr Phe	Ala Ser Ser Glu	Ile Thr Gly	
450	455	460		
aga agt tat ggt ggt	ggt gtt atg aca	ttc gaa cca act	gaa att gga	1440
Arg Ser Tyr Gly Gly	Gly Val Met Thr	Phe Glu Pro Thr	Glu Ile Gly	
465	470	475	480	
gaa atc cta ata cct	tcc ttt gat aac	tta tcc att gat	ttt gat aaa	1488
Glu Ile Leu Ile Pro	Ser Phe Asp Asn	Leu Ser Ile Asp	Phe Asp Lys	
485	490	495		
att gat gcc tta att	cga gaa aag gag	att gaa aaa gtc	ctt gat att	1536
Ile Asp Ala Leu Ile	Arg Glu Lys Glu	Ile Glu Lys Val	Leu Asp Ile	
500	505	510		
gtt gat gaa gct tta	ctt ata aaa tat	cat ggg ttt agt	gag aaa gaa	1584
Val Asp Glu Ala Leu	Leu Ile Lys Tyr	His Gly Phe Ser	Glu Lys Glu	
515	520	525		
gta aaa cag ctt cga	ggg ata tgg aag	aaa ctt tct cag	aga aga aac	1632
Val Lys Gln Leu Arg	Gly Ile Trp Lys	Lys Leu Ser Gln	Arg Arg Asn	

530

535

540

aat aga acg aag aaa taa

1650

Asn Arg Thr Lys Lys

545 550

&lt;210&gt; 2

&lt;211&gt; 549

&lt;212&gt; PRT

&lt;213&gt; Bacillus pumilus

&lt;400&gt; 2

Met Asn Gln Leu Ile Glu Asn Val Asn Leu Gln Lys Leu Arg Gly Gly

1 5 10 15

Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile

20 25 30

Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn

35 40 45

Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn

50 55 60

Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu

65 70 75 80

Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn

85 90 95

Ser Ile Val Asn Ser Asp Phe Phe Gln Phe Val Lys Asp Asn Lys Asn

100 105 110

Lys Lys Phe Asp Thr Ile Ile Gly Asn Pro Pro Phe Ile Arg Tyr Gln

115 120 125

Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu

130 135 140

Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu

145 150 155 160

Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val

165 170 175

Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile

180 185 190

Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys

195 200 205

Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Leu Cys Glu

210 215 220

Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn



225		230		235		240
Leu Asp Gly	Leu Asn Ser Ile Asp Phe	Val Ala Ile Asn Gly	Ser Asn			
	245	250	255			
Val Lys Pro	Ile Glu His Arg Thr Glu	Lys Trp Thr Lys Tyr Phe	Leu			
	260	265	270			
Asn Glu Asp	Glu Ile Leu Leu Leu Gln Ser	Leu Lys Glu Asp Lys	Arg			
	275	280	285			
Val Lys Asn	Cys Asn Asp Tyr Phe Lys Thr	Glu Val Gly Leu Val Thr				
	290	295	300			
Gly Arg Asn	Glu Phe Phe Met Met Lys Glu	Asn Gln Val Lys Glu Trp				
305	310	315	320			
Asn Leu Glu	Glu Tyr Thr Ile Pro Val Thr	Gly Arg Ser Asn Gln Leu				
	325	330	335			
Lys Gly Ile	Thr Phe Thr Glu Asn Asp Phe	His Glu Asn Ser Met Glu				
	340	345	350			
Gln Lys Ala	Ile His Leu Phe Leu Pro Pro	Asp Glu Asp Phe Glu Lys				
	355	360	365			
Leu Pro Ile	Glu Cys Gln Asn Tyr Ile Lys Tyr	Gly Glu Glu Lys Gly				
	370	375	380			
Phe His Gln	Gly Tyr Lys Thr Arg Ile Arg Lys	Arg Trp Tyr Ile Thr				
385	390	395	400			
Pro Ser Arg	Trp Val Pro Asp Ala Phe Ala	Leu Arg Gln Val Asp Gly				
	405	410	415			
Tyr Pro Lys	Leu Ile Leu Asn Glu Thr Asp Ala	Ser Ser Thr Asp Thr				
	420	425	430			
Ile His Arg	Val Arg Phe Lys Glu Gly Ile Asn	Glu Lys Leu Ala Val				
	435	440	445			
Val Ser Phe	Leu Asn Ser Leu Thr Phe Ala Ser	Ser Glu Ile Thr Gly				
	450	455	460			
Arg Ser Tyr	Gly Gly Gly Val Met Thr Phe Glu	Pro Thr Glu Ile Gly				
465	470	475	480			
Glu Ile Leu	Ile Pro Ser Phe Asp Asn Leu Ser	Ile Asp Phe Asp Lys				
	485	490	495			
Ile Asp Ala	Leu Ile Arg Glu Lys Glu Ile Glu	Lys Val Leu Asp Ile				
	500	505	510			
Val Asp Glu	Ala Leu Leu Ile Lys Tyr His Gly	Phe Ser Glu Lys Glu				
	515	520	525			

Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn  
530 535 540

Asn Arg Thr Lys Lys  
545

<210> 3

<211> 3030

<212> DNA

<213> Bacillus pumilus

<220>

<221> CDS

<222> (1)..(3030)

<400> 3

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1 5 10 15

ttt tta aaa cca act tat aat gaa act caa cta agg aat gat ttt ata 96  
Phe Leu Lys Pro Thr Tyr Asn Glu Thr Gln Leu Arg Asn Asp Phe Ile  
20 25 30

gac cca ctt cta aaa tct tta gga tgg gat gtt gat aat acc aaa gga 144  
Asp Pro Leu Leu Lys Ser Leu Gly Trp Asp Val Asp Asn Thr Lys Gly  
35 40 45

aaa aca cat att cta aga gat gtc att caa gaa gaa tac ata gaa ata 192  
Lys Thr His Ile Leu Arg Asp Val Ile Gln Glu Glu Tyr Ile Glu Ile  
50 55 60

aaa gat gag gag aca aag aaa aat cca gat tat aca ctt cgt ata aac 240  
Lys Asp Glu Glu Thr Lys Lys Asn Pro Asp Tyr Thr Leu Arg Ile Asn  
65 70 75 80

ggg acg aga aag ctg ttt gta gag gtt aag aaa ccg tct ttt aat att 288  
Gly Thr Arg Lys Leu Phe Val Glu Val Lys Lys Pro Ser Phe Asn Ile  
85 90 95

ttg aaa tca gct aaa gca gcc ttc caa aca aga aga tat ggt tgg agt 336  
Leu Lys Ser Ala Lys Ala Ala Phe Gln Thr Arg Arg Tyr Gly Trp Ser  
100 105 110

gct aac ctt ggt att tca gta ctt aca aat ttc gag cat cta gtt att 384  
Ala Asn Leu Gly Ile Ser Val Leu Thr Asn Phe Glu His Leu Val Ile  
115 120 125

tat gat tgt aga tat acg cct gac aaa tcc gac aat gaa cat att gct 432  
Tyr Asp Cys Arg Tyr Thr Pro Asp Lys Ser Asp Asn Glu His Ile Ala  
130 135 140

aga tat aaa gtt ttc tct tac gag gaa tat gaa gaa gca ttt gat gaa 480

Arg	Tyr	Lys	Val	Phe	Ser	Tyr	Glu	Glu	Tyr	Glu	Glu	Ala	Phe	Asp	Glu	
145					150					155					160	
ata	aag	gat	ata	att	tca	tat	gag	tca	gcc	aac	tca	ggg	gct	ctg	gac	528
Ile	Lys	Asp	Ile	Ile	Ser	Tyr	Glu	Ser	Ala	Asn	Ser	Gly	Ala	Leu	Asp	
				165					170					175		
gaa	atg	ttt	gat	gta	aat	aca	aga	gtt	ggg	gaa	acg	ttt	gac	gag	tat	576
Glu	Met	Phe	Asp	Val	Asn	Thr	Arg	Val	Gly	Glu	Thr	Phe	Asp	Glu	Tyr	
			180					185					190			
ttt	tta	cag	caa	att	gag	aat	tgg	cgc	gaa	aag	cta	gct	aaa	act	gca	624
Phe	Leu	Gln	Gln	Ile	Glu	Asn	Trp	Arg	Glu	Lys	Leu	Ala	Lys	Thr	Ala	
		195					200					205				
att	aaa	aat	aac	acc	gaa	tta	ggg	gaa	gag	gac	gtc	aat	ttt	att	gtc	672
Ile	Lys	Asn	Asn	Thr	Glu	Leu	Gly	Glu	Glu	Asp	Val	Asn	Phe	Ile	Val	
	210					215				220						
caa	aga	cta	tta	aac	aga	att	att	ttt	ctt	aga	gtt	tgt	gaa	gat	aga	720
Gln	Arg	Leu	Leu	Asn	Arg	Ile	Ile	Phe	Leu	Arg	Val	Cys	Glu	Asp	Arg	
225					230				235					240		
acc	att	gaa	aaa	tat	gaa	aca	att	aaa	agt	ata	aaa	aac	tat	gag	gaa	768
Thr	Ile	Glu	Lys	Tyr	Glu	Thr	Ile	Lys	Ser	Ile	Lys	Asn	Tyr	Glu	Glu	
				245				250					255			
tta	aaa	gat	ctg	ttt	caa	aag	tct	gat	agg	aaa	ttt	aat	tca	ggg	ctc	816
Leu	Lys	Asp	Leu	Phe	Gln	Lys	Ser	Asp	Arg	Lys	Phe	Asn	Ser	Gly	Leu	
			260					265				270				
ttt	gac	ttc	ata	gat	gat	acg	ctc	ttg	ctt	gag	gtt	gaa	att	gat	tcg	864
Phe	Asp	Phe	Ile	Asp	Asp	Thr	Leu	Leu	Leu	Glu	Val	Glu	Ile	Asp	Ser	
	275						280					285				
aat	gta	ttg	ata	gaa	att	ttt	agt	gat	tta	tat	ttc	cca	caa	agc	cca	912
Asn	Val	Leu	Ile	Glu	Ile	Phe	Ser	Asp	Leu	Tyr	Phe	Pro	Gln	Ser	Pro	
	290					295				300						
tat	gat	ttt	tct	gtt	gtc	gat	cca	aca	ata	tta	agc	cag	ata	tat	gaa	960
Tyr	Asp	Phe	Ser	Val	Val	Asp	Pro	Thr	Ile	Leu	Ser	Gln	Ile	Tyr	Glu	
305					310				315					320		
cgt	ttt	cta	ggg	caa	gaa	ata	att	ata	gag	tca	ggg	ggg	aca	ttt	cac	1008
Arg	Phe	Leu	Gly	Gln	Glu	Ile	Ile	Ile	Glu	Ser	Gly	Gly	Thr	Phe	His	
				325				330					335			
att	acg	gag	tca	cca	gaa	gtt	gcg	gcg	tcc	aat	ggg	gtt	gtt	cca	act	1056
Ile	Thr	Glu	Ser	Pro	Glu	Val	Ala	Ala	Ser	Asn	Gly	Val	Val	Pro	Thr	
			340				345					350				
cca	aaa	att	atc	gtc	gaa	cag	ata	gtg	aaa	gac	act	tta	acg	ccc	ctt	1104
Pro	Lys	Ile	Ile	Val	Glu	Gln	Ile	Val	Lys	Asp	Thr	Leu	Thr	Pro</		

370

375

380

ata tgt tgt gga tca gga act ttc cta att tca agt tat gac ttt cta 1200  
Ile Cys Cys Gly Ser Gly Thr Phe Leu Ile Ser Ser Tyr Asp Phe Leu  
385 390 395 400

gta gag aaa gta atg gaa aag ata ata gaa gag aac atc gat gat tca 12